



## ENDOREPLICATION: A MOLECULAR TRICK DURING ANIMAL NEURON EVOLUTION

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### ABSTRACT

*The occurrence of endoreplication has been repeatedly reported in many organisms, including protists, plants, worms, arthropods, molluscs, fishes, and mammals. As a general rule, cells possessing endoreplicated genomes are large-sized and highly metabolically active. Endoreplication has not been frequently reported in neuronal cells that are typically considered to be fully differentiated and non-dividing, and which normally contain a diploid genome. Despite this general statement, various papers indicate that giant neurons in molluscs, as well as supramedullary and hypothalamic magnocellular neurons in fishes, contain DNA amounts larger than 2C. In order to study this issue in greater detail here, we review the available data about endoreplication in invertebrate and vertebrate neurons, and discuss its possible functional significance. As a whole, endoreplication seems to be a sort of molecular trick used by neurons in response to the high functional demands that they experience during evolution.*

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## INTRODUCTION

**E**NDOREPLICATION is a widespread phenomenon observed among protists, plants, and animals, including arthropods, worms, molluscs, and mammals (Edgar and Orr-Weaver 2001). Endoreplicating cells can increase their C values—thereby indicating the DNA contents as a multiple of the normal haploid genome size—as high as 24,000C, for example, as in some plants (Traas et al. 1998). Considering that nuclear and, consequently, cell size are generally proportional to the amount of nuclear DNA, cell growth can be differentially regulated using the ploidy level, thus making endoreplication a common feature in differentiated cells that are large-sized and/or highly metabolically active (Edgar and Orr-Weaver 2001).

Endoreplication has been thoroughly studied in *Drosophila melanogaster*, where many organs (such as the gut, epidermis, fat body, Malpighian tubules, trachea, and salivary glands) initiate endoreplication following the cell-proliferation phase of embryogenesis (White 1973; Smith and Orr-Weaver 1991, Lilly and Duronio 2005; Narbonne-Reveau et al. 2008). These organs continue to endoreplicate during larval development, long after they are fully differentiated. Some adult cells, including ovarian follicle cells, nurse cells, and the sensory neurons in the wing, also endoreplicate, so that the final DNA levels in the larval cells are developmentally programmed (Edgar and Orr-Weaver 2001).

Cell types that undergo endoreplication were also identified in vertebrates, particularly in mammals (Edgar and Orr-Weaver 2001). An example can be found in megakaryocytes that become polyploid up to 128C as a part of their differentiation process in order to increase their ability to bud-off large numbers of platelets (Zimmet and Ravid 2000). A second mammalian cell type that undergoes endoreplication is the trophoblast, which contributes to the placenta and increases its DNA contents up to more than 1000C, presumably to face a high metabolic demand (Varmuza et al. 1988; Zybinska and Zybinska 1996). Some researchers have also reported that the cerebellar Purkinje cells in vertebrates possess endoreplicated ge-

nomes. Indeed, DNA contents higher than 2C were reported for several different species across various studies (e.g., Herman and Lapham 1969; Bohn and Mitchell 1976; Bernocchi and Barni 1985; Del Monte 2006). At the same time, however, other researchers have pointed out that the DNA amount in these cells corresponds to the normal 2C (e.g., Cohen et al. 1973; Mares et al. 1973; Fujita et al. 1974; Fukuda et al. 1978; Mann et al. 1978), therefore making it difficult to draw a definitive conclusion about the real occurrence of endoreplication in this cell type.

For a long time, neurons had been considered “stable cells,” as they are non-dividing and remain 2C throughout their entire lifetime—thus, they are fully differentiated. This view changed in the early 1990s, when it was discovered that the role of neurons in brain repair and brain plasticity appeared to be much more complex and articulated than had been previously expected. Indeed, even though endoreplication had not been frequently reported in neuronal cells, several works proved that giant neurons in molluscs and supramedullary and hypothalamic magnocellular neurons in fishes present DNA contents greater than 2C. Here we review the available data about endoreplication in vertebrate and invertebrate giant neurons, in order to study this issue in more detail.

#### GIGANT ENDOPOLYPLOID NEURONS IN INVERTEBRATES: THE CASE OF GASTROPOD MOLLUSCS

Giant neurons are scattered across several invertebrate species, starting with nematodes and annelids (for a review see Bullock and Horridge 1965), but only in a few cases has the large neuronal size been specifically associated with an increased amount of DNA. A couple of very large neurons (100–120  $\mu\text{m}$ ), the serotonergic Retzius cells of the leech *Hirudo medicinalis*, are probably the first examples of electrophysiologically and biochemically well-characterized giant neurons (Gaskell 1919; Coggeshall 1972). Large-sized neurons are also present in arthropods—such as Diptera, Odonata, Chelicerata, and Arachnoidea—possessing giant cells that have been identified as motor elements (Bullock

TABLE 1

*Suggested mechanisms for genome increase, cell sizes, and maximum DNA content found in molluscs*

Species	Mechanism involved in genome increase	Maximum cell size (diameters)	Maximum DNA amount recorded	References
<i>Achatina fulica</i> **	Partial amplification	28 $\mu\text{m}$ †	128C	Chase and Tolloczko (1987)
<i>Aplysia californica</i> *	Complete polyploidy	1000 $\mu\text{m}$	More than 200,000C	Lasek and Dower (1971)
<i>Helix pomata</i> **	Complete polyploidy	200 $\mu\text{m}$	More than 500C	Manfredi Romanini et al. (1972)
<i>Lymnaea stagnalis</i> **	Complete polyploidy	90 $\mu\text{m}$	4096C	Boer et al. (1977)
<i>Planorbarius corneus</i> **	Partial amplification	80 $\mu\text{m}$	More than 1000C	Lombardo and Sonetti (1977, 1983)
<i>Succinea lauta</i> **	Complete polyploidy	380 $\mu\text{m}$	16,384C	Kirsanova and Anisimov (2000)
<i>Triodopsis divesta</i> **	Complete polyploidy	n. d.	32C	Cowden (1972)

Note:

\*Opisthobranchs.

\*\*Pulmonates.

†At present, the genome size has been evaluated in neurons of 28  $\mu\text{m}$  only.

n. d. = size not determined.

and Horridge 1965), but this does not constitute a rule among invertebrates.

A special case among invertebrates is certainly represented by the occurrence of giant neurons with a DNA content much higher than diploid in the most evolved gastropod molluscs—Opisthobranchia and Pulmonata. These neurons seemingly do not occur in even the primitive Prosobranchia that have been thus far examined (Table 1). In Opisthobranchia and Pulmonata, a clear centralization in the head of the nervous system is constituted by a periesophageal ring of functionally distinct ganglia joined together by nervous connectives and commissures (Figure 1). The giant neurons are found in the central ganglia of each species, located in the cortex layer in constant number and position. Fewer giant neurons occur in the buccal, pleural, and cerebral ganglia, whereas the most occur in the visceral and pedal ganglia. They are intermingled with a heterogeneous population of neurons and glial cells; indeed, even if some diploid nerve cells are small, the majority of neurons occur in a wide range of sizes, and their DNA content directly correlates to nuclear size. Bullock and Horridge (1965) described gastropod giant neurons in their fundamental handbook and stated that they are “extraordinary in

providing the basis of a disproportionate fraction of our knowledge of neuronal cytology, because of the large size and accessibility of some of them” (p. 981). “The largest cells are veritable giants, not only relative to others in the same animals but to the nerve cells in any group of animals and indeed to active cells in general, attaining diameters of 0.8 mm and more” (Bullock and Horridge 1965). Interestingly, these authors also say “they (the large and giant neurons) are especially notable for the size of the nucleus, which is commonly about two-thirds of the diameter of the cell” (Bullock and Horridge 1965:981). Some years later, this occurrence was clearly associated with endoreplication, as shown for the first time by Coggeshall et al. (1970).

The most studied giant gastropod neurons belong to the sea-hare *Aplysia californica*, a marine opisthobranch that became quite well-known through the fundamental studies of Kandel and his group in the 1960s (Frazier et al. 1967). They adopted this animal as a model for investigating the cellular and molecular basis of behavior focusing mainly on the abdominal ganglion, where they identified and mapped all of the single giant and large neurons (about 30) aside from the most prominent

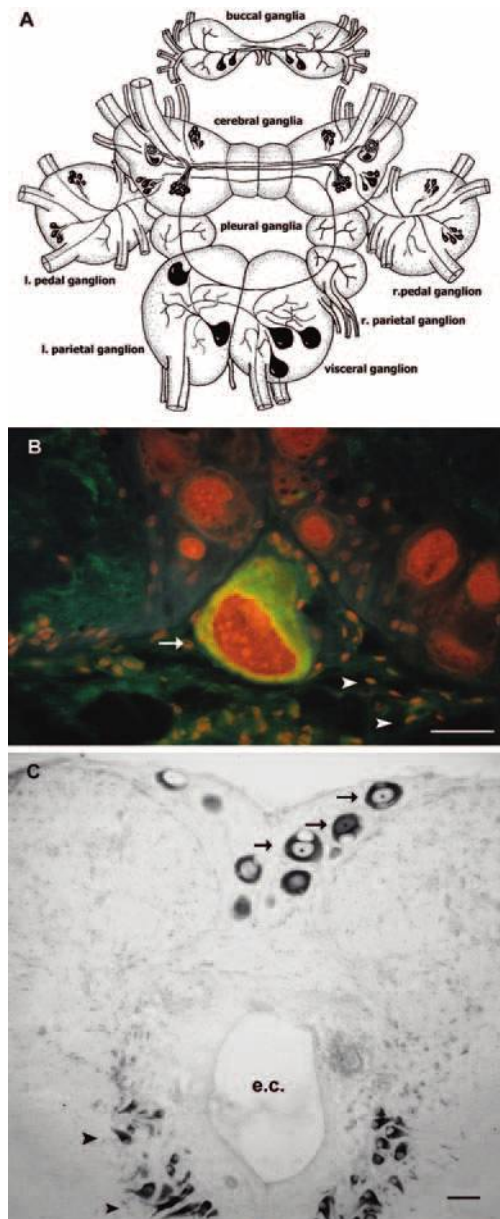


FIGURE 1. GIANT NEURONS IN THE GASTROPOD AND FISH NERVOUS SYSTEMS

(A) Schematic dorsal representation of the centralized nervous system of the gastropod freshwater snail *Planorbarius corneus*, showing some giant neurons (70–100  $\mu\text{m}$  in size) and several clusters of smaller nerve cells localized for their immunopositivity for ACTH. (B) A cross-section through the left parietal ganglion of *P. corneus* showing a giant neuron, as indicated by the arrow, that is positively immuno-fluorescent (FITC fluorophore) for endogenous morphine. The picture shows the nuclei fluorescent in red, stained with propidium iodide. Note the difference in size between the giant neuron nucleus and the much smaller nuclei of glial immunonegative cells, as indicated by the arrowheads. Bar: 50  $\mu\text{m}$ . (C) Transversal section of rostral spinal cord of *Diodon holacanthus* showing the supramedullary neurons clustered (arrows) in the dorsal region as well as the motor neurons (arrowheads). e.c.: ependymal canal. Bar: 100  $\mu\text{m}$ .

cell clusters. The largest neurons they found were named L10 and R2, and were reported to have a soma diameter of almost 1 mm in adults and to contain an ellipsoid nucleus with a long axis of up to 800  $\mu\text{m}$ , which reached a volume of about  $10^6 \mu\text{m}^3$  and was thus visible *in vivo*, even by the naked eye, due to natural pigmentation and refraction. These large, identifiable cells are already well distinguishable in the ganglia of small juveniles specimens and do not change in number; rather, they simply increase in size during the course of the animal's life, in contrast to the small-sized neuron class, which only seems to increase in number.

Through cytophotometric analysis, Coggeshall et al. (1970) found that the DNA contents of *A. californica* giant neurons can vary during development from 2000C to 75,000C by incremental duplications of the whole genome, thus supporting the hypothesis that polyploidy may take place. These data were successively confirmed by fluorimetric analyses, which showed that *A. californica* giant neurons may contain amounts of DNA up to 260,000C times higher than the haploid C value present in spermatozoa, thereby corresponding theoretically to 16–17 complete replications (Lasek and Dower 1971).

The increase in size of the large neurons has been explained as a part of an intrinsic response to an increased functional demand for innervating larger areas of the growing body (Gillette 1991). As Kandel (1976) stated, "Some (neuronal) cell types, often large cells, may never vary in number because they never experience demands for functional elaboration, or if they do they respond [by] enlarging and undergoing DNA replication but not cellular replication" (p. 727). Quantitative analysis of large nuclei performed on Feulgen DNA-stained preparations revealed the occurrence of repeated duplication of the complete genome in other gastropods, viz. the pulmonates *Helix pomatia* (Kuhlman 1969; Manfredi Romanini et al. 1972), *Lymnaea stagnalis* (Boer et al. 1977), *Triodopsis divesta* (Cowden 1972), and *Succinea lauta* (Kirsanova and Anisimov 2000), that prompted a number of further studies supporting the oc-

currence of complete genome duplications in giant neurons (for review see Brodsky and Uryvaeva 1985).

A different situation was found, in contrast, in the giant neurons of the freshwater snail *Planorbarius comeus* and the land snail *Achatina fulica*, where apparently only part of the genome is duplicated (Lombardo et al. 1980; Lombardo and Sonetti 1983; Chase and Tolloczko 1987). Cytochemical and microfluorimetric analyses performed on the nervous system of *P. comeus* were able to distinguish GC-rich from AT-rich DNA strands, and these results suggested that the increase in DNA content could be correlated with an increase in nuclear volume due to a higher differential amplification of GC-rich DNA sequences in specific compartments of the genome. This hypothesis was supported by data reporting that, in *P. comeus*, the increase in nuclear volume is associated with an increase in nucleolus number and in the amount of correspondent perinucleolar chromatin that is generally reported as GC-rich (Lombardo and Sonetti 1977).

As Chase and Tolloczko (1987) observed in *Achatina*, a "differential DNA endoreplication" of some DNA sequences takes place at a higher rate in juvenile specimens, particularly during the period of the animal's greatest growth, but declines rapidly following the onset of sexual maturity. In their microspectrophotometric DNA content determinations, these researchers considered neuronal nuclei with diameters up to 28  $\mu\text{m}$  for technical limits, but they did not encounter discrete size classes corresponding to a simple doubling (Chase and Tolloczko 1987). Furthermore, the analysis of quantitative data reported for measurements of different neuronal size classes and/or different animal age/size classes in *Planorbarius* and *Achatina* suggested that complete genome replication and differential amplification of specific sequences could both run in concert, thus keeping the two processes coupled or unmatched. It could be very interesting to revisit these data using new molecular techniques, such as the Real Time PCR, in order to experimentally test such a hypothesis.



Functional explanations supporting the differential amplification of parts of the genome in molluscs might entail genes involved in the synthesis of particular molecules, proteins, or RNAs that are required for a rapid biosynthesis of specific gene products. Alternatively, the amplified genes might be involved in a more aspecific mechanism—for instance, the production of a considerable amount of rRNA aimed to increase the protein synthesis capacity. Picciotto et al.'s (1986) investigations of *A. californica* seem to exclude a differential amplification of genes that encode specific neuropeptides.

#### GIANT ENDOPOLYPLOID NEURONS IN TELEOSTS

With regard to extra DNA content in teleost neurons, endoreplication phenomena were suggested in the hypothalamic magnocellular neurons in the preoptic and tuberal complexes (Benedetti et al. 1999) of the angler fish *Lophius piscatorius* and in the long-spine porcupinefish *Diodon holacanthus*. Computerized image analysis on histological sections treated with Feulgen reaction showed a correlation between the increase in nuclear area of the neurosecretory neurons and the increase in their Feulgen-DNA content. Microfluorimetric analysis on slides treated with ethidium bromide staining demonstrated that the hypothalamic neurons have nuclei whose DNA content increases with an increase in their size, reaching values of about 68C in *L. piscatorius* and about 84C in *D. holacanthus* (Benedetti et al. 1999). Moreover, the analysis of C values and percent distributions of hypothalamic neuron nuclei suggested that the increase in DNA amounts might be due to differential gene amplification (Benedetti et al. 1999).

The most unexpected finding involves DNA endoreplication occurrence in teleost supramedullary neurons (SN), which are giant neurons composing a particular neuronal group belonging to the autonomic nervous system (Mola and Cuoghi 2004). In the pufferfish *Takifugu niphobles*, SN-free nerve endings were detected in the skin near mucous glands (Funakoshi et al. 1998); consequently, SN were thought to act as

mucous secretion agents in fish chemical defenses against parasites or predators (Zottoli et al. 1999).

SN are located on the dorsal surface of the spinal cord of various species of teleosts. In Clupeiformes, Syngnathiformes, Scorpaeniformes, Pleuronectiformes, and Perciformes orders, SN are aligned along the spinal cord, whereas they are clustered at the rostral spinal cord in Tetraodontiformes (Figure 1C), Lophiiformes, and Batrachoidiformes (for a complete review, see Mola and Cuoghi 2004). Although ultrastructural features are quite similar for SN belonging to all the different species and orders examined up until now, SN morphology, number, and size are unique to each species (Mola and Cuoghi 2004). For instance, *Solea ocellata* SN have recently been demonstrated as a transitional form, neither singularly aligned nor authentically clusterized, but instead forming small groups of two or three SN alternate to singular SN (Cuoghi and Mola 2007). These morphological observations give us descriptive data, but also furnish functional information, since only clustered SN (Sassi et al. 1995; Mola et al. 2001) and *S. ocellata* SN (Cuoghi and Mola, 2007) have a supra-normal DNA amount.

Feulgen reaction and fluorimetric analyses performed on nuclei of clustered SN of *D. holacanthus* and *L. piscatorius* indicated that the DNA amount is a multiple of the normal diploid quantity (2C), and it is always matched with the sizes of the nucleus, cell, and animal. In particular, DNA content in *D. holacanthus* SN can reach more than 500C (Mola et al. 2001), and even 5000C in *L. piscatorius* specimens (Sassi et al. 1995). Also, clustered SN in *Tetraodon fluviatilis* have a DNA content higher than 2C (Cuoghi, unpublished data). Accordingly, recent results for *S. ocellata* SN, obtained with cytofluorimetric evaluation, indicated DNA content ranging from 6C in smaller SN to 100C in larger SN (Cuoghi and Mola 2007) (Table 2). It can therefore be inferred that DNA supra-normal content is a common characteristic of all clustered SN.

The DNA amplification in *D. holacanthus*

TABLE 2  
Disposition, number, and DNA content of giant neurons in fish

Species	SN disposition	Total SN number	SN size (medium diameters)	Nucleus size (medium diameters)	Maximum DNA amount recorded	2C value
<i>Lophius piscatorius</i>	Clusterized	More than 200	90 × 105 μm	35 × 40 μm	More than 5000 C	2,04 pg
<i>Diodon holacanthus</i>	Clusterized	200	65 × 75 μm	35 × 45 μm	More than 500 C	1,56 pg
<i>Solea ocellata</i>	Small groups	70	120 × 80 μm	60 × 40 μm	100 C	1,46 pg

Note: Disposition, total number, cell and nucleus sizes, and maximum DNA content of supramedullary neurons found in the three different teleost species examined, with 2C values for each species.

and *L. piscatorius* SN does not take in the complete genome, but occurs only for specific genes (Sassi et al. 1995; Mola et al. 2001). Nucleolar organizing region (NOR) staining, carried out with the aim of testing the involvement of NORs in the growth of nucleolar area, showed that each neuron in a cluster contains a large nucleolus. The nucleolar ultrastructural data, together with silver NOR staining, suggested an intense production of ribosomal components (Sassi et al. 1995; Cuoghi and Marini 2001); indeed, it is generally accepted that silver staining reveals transcriptional activity of ribosomal genes (Howell 1977; Hubbel 1985), or at least the transcriptional potential of such genes (Sumner 1990). The clustered SN, as in those of *S. ocellata* as well, are giant cells (larger than the aligned SN) with high metabolic rates, as suggested by ultrastructural features (Cuoghi 2001; Cuoghi and Marini 2001) and by cytochemical tests that demonstrate the presence of multiple signaling molecules, such as noradrenaline, ACTH-like peptide, CCK-like peptide, and nitric oxide (Mola and Cuoghi 2004; Cuoghi and Mola 2007). Therefore, we hypothesize that clustered SN (including those of *S. ocellata*) developed high DNA contents through an increased production of ribosomes in order to satisfy their high metabolic rates. Accordingly, the data on hypothalamic neurosecretory neurons in *L. piscatorius* and *D. holacanthus* indicate that a marked increase of DNA content is not an exclusive feature of SN, as the two cell types share a large size and an intense biosynthetic activity (neurohormones and neurotransmitters, respectively).

What genome regions are amplified in clustered SN? There is not one simple answer to this question. Chromomycin A<sub>3</sub> (CMA<sub>3</sub>) and 4',6'-Diamino-2-phenylindole (DAPI) staining followed by microfluorimetric evaluations suggested that, in *L. piscatorius*, the amplification occurs with regard to GC-rich sequences (Sassi et al. 1995), but the same does not hold true for *D. holacanthus* SN (Mola et al. 2001). Even if staining with fluorochromes presents several limitations, this discrepancy does not seem to be related to the fluorimetric method, but could instead be related to genes that are passively amplified simply due to their proximity to the important genes. In other words, the difference in results obtained for two phylogenetically distinct fish species could simply reflect differences in their genomic organization.

#### CONCLUSIONS AND PERSPECTIVES

Fluorimetric analyses reported the occurrence of endoreplication in neurons of phylogenetically unrelated species; it is not easy, therefore, to provide a unique explanation for the role of endoreplication during evolution. The use of microfluorimetric measurements cannot furnish a very accurate measure of the degree of endoreplication that can be evaluated using genomic approaches, thus bringing with it a risk of over-estimation in the degree of endoreplication, but providing convincing evidence of endoreplication in neurons nonetheless, so that the occurrence of endoreplication rather than its estimate can be properly discussed.

In this regard, at least two generalizations can be made according to previous

papers (Edgar and Orr-Weaver 2001; Anisimov 2005). First, endoreplication seems to be typical for large, metabolically active cells, suggesting that it is an effective tool for allowing cells to increase their mass or metabolic output (Edgar and Orr-Weaver 2001). Second, since endoreplication permits growth without periodic rearrangements of cytoskeletal elements or cell-cell contacts, as occur in mitosis, it may cause relatively little disruption to the structure of a differentiated tissue. Together, these properties make endoreplication an advantageous strategy for cells and tissues that, although strongly differentiated, still must continue to grow (Edgar and Orr-Weaver 2001). These features are extremely important to neurons that are fully differentiated, non-dividing cells because they could increase their functional plasticity without disrupting the structure of the nervous system.

The analysis of endoreplication phenomena in neurons allows us to obtain some insight into its frequency. First, it should be emphasized that giant or large-sized neurons, although found in a wide variety of invertebrate and vertebrate species, generally do not occur very frequently. If the occurrence of endoreplication is considered from an evolutionary perspective, it seems to have been “re-invented” several times. An important question is: why do giant neurons choose endoreplication? That is, why do neurons belonging to particular systems become giant cells with very high DNA contents and impressive “metabolic machines” instead of forming more numerous, normally diploid neurons during development? For these cells, endoreplication must be a very useful and economically profitable way to overcome high metabolic demands, as it is present in species phylogenetically distant from one another, and has therefore arisen independently, more than once, during evolution.

As suggested by Anisimov (2005), the single giant neuron could be considered an endoclone, functionally equivalent to cell clones (or to its part). We hypothesize that the presence of a single giant cell may be considered an alternative to the multi-

ple cell system; in other words, a giant or large neuron could be regarded as a “single cell ganglion” combining the properties of many equivalent cells. This evolutionary trick could be useful from a functional point of view as well, since it allows an increase in cell functionality without affecting the tissue structure or its organization. Moreover, the presence of a single endoreplicated neuron could facilitate the functionality of the brain, since a single cell can work without the coordinating system that is necessary in a multiple cell system.

On the other hand, the presence of a unique, multifunctional, and highly active neuron could represent an important weak point in the nervous system, as it makes it more susceptible to damage in comparison to a multiple cell system in which individual damaged or aged cells might be replaced relatively easily. Moreover, a system made up of several cells could be more efficient than a single large neuron in terms of assuring integration of the afferent signals and the successive intracellular transduction of these signals into a more accurate final output.

Data on endoreplication in neurons suggest that it can be obtained by repeated rounds of specific gene amplification or by a complete genome replication. For instance, in the molluscs *A. californica*, *H. pomatia*, and *L. stagnalis*, neuronal DNA contents vary by incremental duplications of the whole genome, whereas in the land snail *A. fulica*, in the planorbid *P. corneus*, and in all endoploid vertebrate neurons studied, a differential amplification of specific DNA sequences has been reported. This difference could be due to the absence of clustering in amplicons of those DNA sequences whose amplification is necessary in order to satisfy neuron functional requirements in *A. californica*, *H. pomatia*, and *L. stagnalis*. The presence of a DNA endoreplication consisting of rounds of amplification of specific gene sequences instead of one complete genome replication represents an evolutionary advantage, as the amplification of unnecessary DNA sequences can be avoided.



A further discrepancy seems to be related to the observed differences in the amplified genomic compartments. For example, *L. piscatorius* and *P. cornutus* amplify predominantly GC-rich DNAs, whereas *D. holacanthus* and some molluscs do not show any preferential AT- or GC-rich DNA amplification. In this regard, the availability of techniques such as FACS could lead to better evaluations and, in turn, interesting data that could then be analyzed at a genome level with quantitative methods (like Real Time PCR) in order to identify the DNA sequences that have been amplified in each species. However, the reported discrepancy in results is not necessarily controversial, as amplicons can consist of genes whose proteins are essential for cells and DNA sequences that “go along for the ride” because of their association with other functionally important genes. This endoreplication feature suggests that the differential amplification that occurs in *L. piscatorius* and *D. holacanthus* does not reflect different functions of SN, but only a difference in the type of DNA sequences that are amplified passively because of their proximity to the functionally important ones. This result could simply be due to differences in genome organization that occur in the two phylogenetically distant fish species that were studied, and a similar hypothesis could explain the data observed in molluscs as well.

At the same time, the discontinuous presence of DNA endoreplication in neurons at a phylogenetic level leads us to suggest that endoreplication has been independently “re-invented” several times during both mollusc and teleost evolution. This also supports the hypothesis that the increase in DNA content may be due to different mechanisms that, in some species, lead to replication of the whole genome but, in others, bring about specific gene amplification (Sun and Deng 2005; Sun et al. 2008). Interesting evidence

for this can be seen in the two species of puffer fish whose genomes have been sequenced (Jaillon et al. 2004), thus making puffers useful models for the study of endoreplication in neurons. Also, the availability of the whole sequenced genome of the mollusc *A. californica* (<http://www.broadinstitute.org/science/projects/mammals-models/vertebrates-invertebrates/aplysia/aplysia-genome-sequencing-project>) provides us with the opportunity to study endoreplication from an evolutionary point of view through the comparison of molluscs and puffers. Furthermore, the availability of different EST libraries, both in puffers and molluscs, could furnish important data for identifying genes that undergo endoreplication, thereby allowing us to more accurately define the precise functional roles of endoreplication in neurons.

Despite the available results, a number of substantial and intriguing questions still remain. For instance, which genetic programs mediate the switch to endoreplication? What programs define which cell types will undergo endoreplication? How frequently did novel mechanisms for endoreplication arise during evolution? Answers to these questions may provide new and important insights into cell differentiation and functioning that pertain not only to endoreplication, but to the mechanisms used in proliferation cycles and the growth control of neurons as well. Genomic and transcriptomic analyses could bring about future improvements in our understanding of endoreplication in neurons, providing us with new perspective on the role it plays in the nervous system.

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